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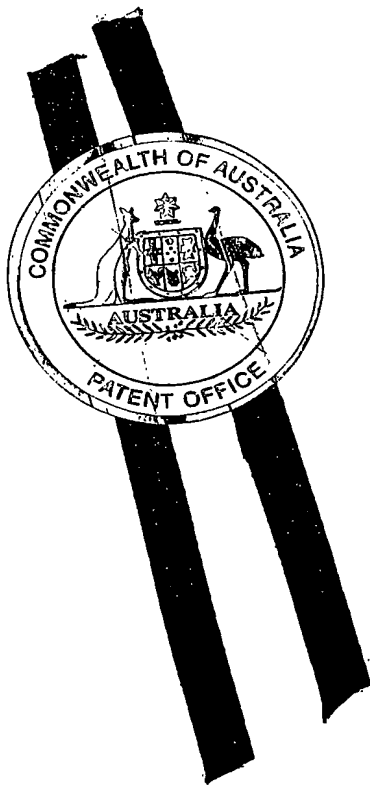
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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002953393 for a patent by GARVAN INSTITUTE OF MEDICAL RESEARCH as filed on 16 December 2002.



WITNESS my hand this
Seventh day of January 2004

J R Yabsley

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Patents Act 1990

Garvan Institute of Medical Research

PROVISIONAL SPECIFICATION

Invention Title:

*Methods of treatment of feeding disorders and disorders of glucose uptake or
metabolism and identifying therapeutic reagents therefor*

The invention is described in the following statement:

Methods of treatment of feeding disorders and disorders of glucose uptake or metabolism and identifying therapeutic reagents therefor

Field of the invention

5 [0001] The present invention relates generally to the treatment of feeding disorders and disorders of glucose uptake or metabolism, such as, for example, diabetes, obesity, anorexia or bulimia, in humans and other animals. More particularly, this invention provides methods of modifying adipose tissue (e.g., in connection with
10 with treating overeating, bulimia or anorexia). The invention also relates to method for identifying modulators of glucose uptake or metabolism that are useful in the therapeutic methods described herein, e.g. using a non-human animal model.

Background to the invention

1. General

15 [0002] This specification contains nucleotide and amino acid sequence information prepared using PatentIn Version 3.1, presented herein after the claims. Each nucleotide sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, <213> etc). The length and type of sequence (DNA, protein (PRT), etc), and source organism for each
20 nucleotide sequence, are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide sequences referred to in the specification are defined by the term "SEQ ID NO:", followed by the sequence identifier (eg. SEQ ID NO: 1 refers to the sequence in the sequence listing designated as <400>1).

25

[0003] The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M
30 represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a

nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

5 [0004] As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

10 [0005] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

15 [0006] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

20 [0007] The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

25 [0008] The present invention is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, peptide synthesis in solution, solid phase peptide synthesis, and immunology. Such procedures are described, for example, in the following texts that are incorporated by reference:

- 30 1. Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Second Edition (1989), whole of Vols I, II, and III;

2. DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL Press, Oxford, whole of text;
3. Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed., 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp1-22; Atkinson *et al.*, pp35-81; Sproat *et al.*, pp 83-115; and Wu *et al.*, pp 135-151;
4. Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text;
5. Animal Cell Culture: Practical Approach, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970, whole of text;
6. Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text;
7. Perbal, B., A Practical Guide to Molecular Cloning (1984);
8. Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series;
9. J.F. Ramalho Ortigão, "The Chemistry of Peptide Synthesis" *In: Knowledge database of Access to Virtual Laboratory website (Interactiva, Germany);*
10. Sakakibara, D., Teichman, J., Lien, E. Land Fenichel, R.L. (1976). *Biochem. Biophys. Res. Commun.* 73 336-342
11. Merrifield, R.B. (1963). *J. Am. Chem. Soc.* 85, 2149-2154.
12. Barany, G. and Merrifield, R.B. (1979) in *The Peptides* (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York.
13. Wünsch, E., ed. (1974) *Synthese von Peptiden in Houben-Weyls Methoden der Organischen Chemie* (Müller, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart.
14. Bodanszky, M. (1984) *Principles of Peptide Synthesis*, Springer-Verlag, Heidelberg.
15. Bodanszky, M. & Bodanszky, A. (1984) *The Practice of Peptide Synthesis*, Springer-Verlag, Heidelberg.
16. Bodanszky, M. (1985) *Int. J. Peptide Protein Res.* 25, 449-474.
17. Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications).

2. Description of the related art

[0009] The c-Cbl protein is a multi-adaptor protein that is involved in ligand-induced down regulation of receptor tyrosine kinases. The interaction between c-Cbl and its many binding partners involves particular c-Cbl protein domains. All Cbl proteins have a high degree of sequence homology between their tyrosine kinase-binding, linker and RING finger domains, and most have extensive proline-rich regions in their carboxy-terminal halves. The tyrosine kinase-binding domain is composed of three interacting domains comprising a four-helix bundle, a Ca^{2+} -binding EF hand, and an atypical or variant Src homology region 2 (SH2) domain that is connected to the RING finger by a short linker domain. A ubiquitin-associated (UBA)/LZ domain at the carboxyl terminus of c-Cbl, Cbl-b and D-Cbl has homology to known ubiquitin-associated domains and leucine zippers and is believed to be involved in Cbl-mediated ubiquitination of active receptors, which is essential for receptor degradation and turnover, thereby leading to cessation of downstream signalling from the receptor (Soubeyran *et al.*, *Nature* 416, 183-187, 2002).

15

[0010] Diabetes, and conditions related thereto such as obesity, are major health concerns throughout the world, and contribute to morbidity and mortality. Non-insulin dependent diabetes mellitus (NIDDM or type II diabetes), is the major form of diabetes in developed countries, however efficient means of therapeutic intervention are lacking. While a large number of environmental and genetic factors contribute to the risk of NIDDM in the United States, prolonged obesity is by far the largest risk factor.

[0011] Before the development of diabetes, many obese patients develop a peripheral resistance to the actions of insulin. The molecular basis of insulin-resistance in obesity has been the subject of intensive study, by nonetheless remains elusive. Insights into components and mechanisms of the link between obesity and insulin resistance have been gained from mouse models of obesity which display obesity-induced insulin resistance. The molecular basis of the various mouse obesity models covers a range of mechanisms; nonetheless these all develop diabetes, either before or after the onset of obesity.

30

[0012] Obesity in humans and rodents is also commonly associated with insulin resistance in fat and muscle cells (LeRoith *et al.*, *Diabetes Mellitus: a Fundamental*

and Clinical Text. (Lippincott-Raven, Philadelphia, 1996); DeFronzo et al., *Diabetes Care* 15:318-68 (1992); Rifkin et al., *Diabetes Mellitus*, (Elsevier, N.Y., 1990)).

- [0013] Much work has focussed on the insulin-sensitive glucose transporter GLUT4
- 5 (Watson and Pessin, *Exp. Cell Res.* 271: 75-83, 2001; Bogan et al. published U.S. Patent Application No. 20020052012). Insulin binds to the insulin receptor (IR) in the plasma membrane, where it activates tyrosine kinase in a cascade of events involving phosphatidylinositol 3-kinase (PI 3-K)-mediated recruitment of GLUT4 to the cell surface Min et al., *Mol. Cell.* 3: 751-760, 1999; Olson et al., *Mol. Cell. Biol.* 17: 2425-
- 10 2435, 1997; Hausdorff et al., *J. Biol. Chem.* 270: 12965-12968, 1995; Elmendorf et al., *J. Biol. Chem.* 273: 13289-13296, 1998; Holman et al., *J. Biol. Chem.* 265: 18172-18179, 1990; Piper et al., *Am. J. Physiol.* 260: C570-C580). The activation of PI 3-K However, PI-K3-mediated trafficking of GLUT4 is not sufficient to explain the extent of insulin resistance (Pessin et al., *J. Clin. invest.* 106: 165-169, 2000). For example,
- 15 other growth factors and adhesion molecules that can activate PI 3-K and its downstream kinases (ie. AKT, protein kinase C ζ / λ (PKC ζ / λ)) have no effect on glucose transport or GLUT4 translocation. Additionally, mutant mice lacking GLUT4 develop only mild hyperinsulinemia (Katz et al., *Nature* 377:151-155, 1995).
- 20 [0014] A second PI 3K-independent signalling cascade, initiated by recruitment of c-Cbl (Langdon et al., *J. Virol.* 63: 5420-5424, 1989; Langdon et al., *Proc. Natl Acad. Sci. USA* 86: 1168-1172, 1989) to the insulin receptor, has been postulated to be involved in insulin-stimulated glucose transport and uptake in fat and muscle cells. In this pathway, the c-Cbl protein is also recruited to the insulin receptor by interaction
- 25 with the adaptor protein CAP, through one of three SH3 domains in the carboxyl-terminus of CAP. c-Cbl is then phosphorylated by the receptor, and the CAP-Cbl complex dissociates from the insulin receptor and moves to a caveolin-enriches triton insoluble membrane fraction. Based upon two-hybrid assay data measuring *in vitro* protein-protein associations Baumann et al., *Nature* 407: 202-207, 2000, showed that
- 30 c-Cbl forms a ternary complex with two other proteins, CAP and flotillin. The interaction with flotillin directs the CAP-Cbl complex to the lipid raft sub-domain of the plasma membrane. Baumann et al. *Nature* 407: 202-207, 2000, also showed that both insulin-stimulated glucose transport and GLUT4 translocation to the cell surface are attenuated by about 50% in 3T3-L1 adipocytes by expression of a truncated CAP

protein lacking SH3 domains (i.e. CAP Δ SH3). If this pathway were to operate in fat cells and/or muscle cells *in vivo*, it would be expected that insulin-induced glucose uptake and its subsequent incorporation into both glycogen and lipid would be impaired in situations which disrupt formation or activity of the c-Cbl-CAP-flotillin complex.

[0015] An interaction between c-Cbl and APS, an adaptor protein having a PH domain and SH2 domain (Ahmed et al., Biochem. J. 341, 665-668, 1999) is required for c-Cbl to bind to the insulin receptor in the caveolae-small invaginations in the plasma membrane that are a subset of the lipid raft domains. In this pathway, APS interacts with the phosphorylated insulin receptor via its SH2 domain, and subsequently undergoes tyrosine phosphorylation at a specific residue in the C-terminus of the protein. Upon phosphorylation, APS recruits c-Cbl to the receptor through an atypical SH2 domain of c-Cbl (Saltiel and Pessin, TRENDS Cell Biol. 12, 65-71, 2002).

[0016] Based upon co-localization studies, Chiang et al., Nature 410: 944-948, 2001 also showed that the time-course for the insulin-stimulated migration of c-Cbl parallels movement of the SH2-containing adaptor protein Crkl, and the guanyl nucleotide exchange factor C3G, into the caveolin-enriched triton insoluble membrane fraction. Chiang et al. also demonstrated that C3G exchanges GTP for GDP on TC10, a Rho-family GTP-binding protein that regulates GLUT4 transport. In this pathway, phosphorylated c-Cbl recruits Crkl to the lipid rafts, along with C3G to facilitate activation of TC10 by C3G (Chiang et al., Nature 410: 944-948, 2001).

[0017] No direct role for c-Cbl in modulating glucose uptake or GLUT4 translocation has been demonstrated in muscle and fat cells *in vivo*. This is because the binding studies by Baumann et al. and Chiang et al. *supra* were carried out in isolated 3T3L1 adipocytes and do not suggest that equivalent effects occur *in vivo*, let alone in both muscle and fat cells of animals. It is also unclear whether the effects reported by Baumann et al. for the over expression of CAP Δ SH3 protein in isolated adipocytes were a direct consequence of CAP failing to bind c-Cbl or to a secondary effect of expressing the mutant CAP Δ SH3 protein. For example, the expressed CAP Δ SH3 protein may have modified the ability of endogenous CAP to bind other proteins in the lipid rafts or elsewhere in the cell. Over expression of the CAP Δ SH3 protein also

decreases insulin-stimulated recruitment of C3G into lipid rafts, and reduces the basal level of activated TC10 in 3T3-L1 adipocytes (Chiang et al., Nature 410: 944-948, 2001). Accordingly, no clear direct role has emerged for c-Cbl in modulating glucose uptake and incorporation into lipid in both fat cells and muscle cells of animals. Thus,
 5 it is not possible at present to conclude that impaired c-Cbl-CAP-flotillin complex formation and activity are sufficient to produce insulin resistance in humans and other animals.

[0018] A number of mouse models have been developed having genetic obesity-
 10 diabetes syndromes (Herberg, et al., Metabolism 26: 59-99, 1977). The mice typically have hyperglycemia, hyperinsulinemia, and obesity, albeit to different degrees, with different times of onset, and for different reasons. In the yellow obese mouse ($A^{y/a}$), a dominant mutation causes the ectopic, ubiquitous expression of the agouti protein, resulting in a condition similar to adult-onset obesity and non-insulin-dependent
 15 diabetes mellitus (Michaud et al., Proc Natl Acad Sci USA 91: 2562-2566, 1994). Obese (ob/ob) (Zhang et al., Nature 372: 425-432, 1994); diabetes (db/db) (Tartaglia et al., Cell 83: 1263-1271, 1995), adipose (cpe/cpe) (Naggert et al., Nat. Genet. 10: 135-142, 1995) and tubby (tub/tub) (Kleyn et al., Cell 85: 281-290, 1996; Noben-Trauth et al., Nature 380: 534-548, 1996) are mutations in the genes encoding leptin,
 20 the leptin receptor, carboxypeptidase E, and a member of a new family of genes encoding tubby-like proteins, respectively. Obese mice exhibit hyperglycemia, glucose intolerance, and elevated plasma insulin, which develops after the onset of obesity. In db/db mice, elevation of plasma insulin occurs at 2 weeks of age, preceding the onset of obesity at 3-4 weeks and elevation of blood glucose levels at 4-8 weeks. Adipose
 25 mice have hyperinsulinemia throughout life in association with hypertrophy and hyperplasia of the islets of Langerhans, with transient hyperglycemia. Tubby mice have normal blood glucose, however plasma insulin is elevated prior to obvious signs of obesity, and the islets of Langerhans are enlarged.

30 [0019] Mouse models having impaired glucose uptake are highly desirable. By virtue of examining their phenotype, such models would have utility in determining appropriate targets for the therapy of a wide range of disorders associated with aberrant glucose metabolism and for determining the efficacy or specificity of therapeutics.

Summary of the invention

[0020] In work leading up to the present invention, the inventors studied the role of Cbl, particularly c-Cbl, in insulin resistance, obesity and diabetes *in vivo*. Surprisingly, the inventors found that a targeted disruption of a Cbl locus in mice sufficient to prevent functional Cbl protein from being expressed, does not disrupt, has little or no defect on basal or insulin-induced glucose uptake and incorporation of glucose into lipid in adipocytes. In muscle cells, Cbl-deficient mice were found to have elevated basal glucose uptake, consistent with an enhanced metabolic rate. Cbl-deficient mice had significantly smaller adipocytes than wild-type counterparts of the same approximate weight. Consistent with this conclusion, the Cbl-deficient mice also had an elevated temperature relative to normal mice, suggesting that the enhanced ratio of lean muscle mass to body fat in Cbl-deficient mice is a consequence of enhanced metabolic rate. It was also surprising that the feeding behaviour of the animals was markedly modified, as Cbl-deficient mice exhibited a markedly enhanced appetite as determined by the amount of food consumed per day (dietary intake). These data also indicate that Cbl, such as, for example, c-Cbl, is directly involved in modulating the feeding behaviour of animals and in regulating fat deposition in adipocytes in mammals. The Cbl protein and Cbl-deficient mouse model are therefore useful for identifying compounds that specifically modulate a Cbl-mediated characteristic such as, for example, feeding behaviour (e.g. in the treatment of anorexia or bulimia), fat deposition, metabolic rate, the ratio of lean muscle mass to body fat, or glucose uptake (e.g. in the treatment of obesity or type II diabetes). These modulators are identified by screening animals for their effect on the phenotype of the mouse model, or alternatively, by directly screening the Cbl protein for altered function, including but not limited to any specific known functions of Cbl such as, for example, binding activity, ubiquitin ligase activity, or by screening for the effect of the compound on the extent of tyrosine phosphorylation of Cbl.

[0021] Accordingly, one aspect of the present invention provides a method of identifying a compound that suppresses or reduces feeding behaviour, such as, for example, in the treatment of obesity, said method comprising: (a) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces

or prevents expression of functional Cbl in said animal; and (b) determining the feeding behaviour of the animal, wherein reduced appetite or dietary intake of the animal compared to the appetite or dietary intake of a Cbl-deficient animal to which the compound has not been administered indicates that the compound suppresses or
5 reduces feeding behaviour.

[0022] In an alternative embodiment, the present invention provides a method for identifying a compound that suppresses or reduces feeding behaviour, such as, for example, in the treatment of obesity, said method comprising determining the ubiquitin
10 ligase activity of a Cbl protein in the presence and absence of the compound wherein enhanced ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound suppresses or reduces feeding behaviour. In an alternative embodiment, the present invention provides a method for identifying a compound that suppresses or reduces feeding behaviour comprising determining the
15 level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein enhanced phosphorylation of tyrosine residues in the Cbl protein in the presence of the compound indicates that the compound suppresses or reduces feeding behaviour.

[0023] Another embodiment of the invention provides a method of identifying a compound that enhances feeding behaviour, such as, for example, in the treatment of anorexia or bulimia, said method comprising: (a) administering a compound that suppresses appetite or dietary intake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic
25 modification reduces or prevents expression of functional Cbl in said animal and determining the feeding behaviour of the animal; (b) administering a compound to the animal and determining the feeding behaviour of the animal, wherein enhanced appetite or dietary intake at (b) compared to (a) indicates that the compound enhances feeding behaviour.

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[0024] In an alternative embodiment, the invention provides a method of identifying a compound that enhances feeding comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein reduced

ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound enhances feeding behaviour.

[0025] In an alternative embodiment, the present invention provides a method for
5 identifying a compound that enhances feeding behaviour comprising determining the level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein reduced phosphorylation of tyrosine residues in the Cbl protein in the presence of the compound indicates that the compound enhances feeding behaviour.

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[0026] In an alternative embodiment, the invention provides a method of identifying a compound that modulates feeding behaviour, such as, for example, in the treatment of anorexia or bulimia, said method comprising: (a) administering a compound to a non-human animal expressing a functional Cbl protein and determining the feeding
15 behaviour of the animal; (b) determining the feeding behaviour of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (c) comparing the feeding behaviour of the animals at (a) and (b) wherein a comparable feeding behaviour between (a) and (b) indicates
20 that the compound modulates feeding behaviour.

[0027] In an alternative embodiment, the invention provides a method of identifying a compound that modulates feeding comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein modified
25 ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound enhances feeding behaviour.

[0028] In an alternative embodiment, the present invention provides a method for identifying a compound that modulates feeding behaviour comprising determining the
30 level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein modified phosphorylation of tyrosine residues in the Cbl protein in the presence of the compound indicates that the compound modulates feeding behaviour.

[0029] A further aspect of the present invention provides a method of identifying a compound that enhances fat deposition or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate such as, for example, in the treatment of hypolipidemia (e.g. induced by HIV antiviral or anti-cancer therapeutic),
5 said method comprising: (a) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (b) determining the fat content of the animal, wherein enhanced fat content of the animal compared to the fat content of a Cbl-deficient animal to which the
10 compound has not been administered indicates that the compound enhances fat deposition or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate.

[0030] In an alternative embodiment, the present invention provides a method of
15 identifying a compound that enhances fat deposition or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein enhanced ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound enhances fat deposition or
20 reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate.

[0031] In an alternative embodiment, the present invention provides a method for identifying a compound that enhances fat deposition or reduces lean muscle mass or
25 enhances the ratio of body fat to muscle or reduces metabolic rate comprising determining the level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein enhanced phosphorylation of tyrosine residues in the Cbl protein in the presence of the compound indicates that the compound enhances fat deposition or reduces lean muscle mass or enhances the ratio of body
30 fat to muscle or reduces metabolic rate .

[0032] In an alternative embodiment, the invention provides a method of identifying a compound that reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate, such as, for example, in the

treatment of obesity or neurodegenerative disorders or for cosmetic purposes such as bodybuilding or weight loss, said method comprising: (a) administering a compound that enhances fat deposition or glucose uptake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said
5 genetic modification reduces or prevents expression of functional Cbl in said animal and determining the fat content of the animal; (b) administering a compound to the animal and determining the fat content of the animal, wherein a similar or reduced fat content at (b) compared to (a) indicates that the compound reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances
10 metabolic rate.

[0033] In an alternative embodiment, the invention provides a method of identifying a compound that reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate comprising: (a) administering a
15 compound to a non-human animal expressing a functional Cbl protein and determining the fat content of the animal; (b) determining the fat content of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (c) comparing the fat contents of the animals at (a) and (b) wherein a
20 comparable fat content between (a) and (b) indicates that the compound reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate.

[0034] In an alternative embodiment, the present invention provides a method of
25 identifying a compound that reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein reduced ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound reduces fat deposition or
30 enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate.

[0035] In an alternative embodiment, the present invention provides a method for identifying a compound that reduces fat deposition or enhances lean muscle mass or

reduces the ratio of body fat to muscle or enhances metabolic rate comprising determining the level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein reduced phosphorylation of tyrosine residues in the Cbl protein in the presence of the compound indicates that the compound reduces fat
5 deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate .

[0036] A further aspect of the present invention provides a method of identifying a compound that enhances glucose uptake such as, for example, in the treatment of
10 hypolipidemia (e.g. induced by HIV antiviral or anti-cancer therapeutic), said method comprising: (a) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (b)
15 determining the glucose uptake into liver, fat or muscle cells of the animal, wherein enhanced uptake compared to the glucose uptake into liver, fat or muscle cells of a Cbl-deficient animal to which the compound has not been administered indicates that the compound enhances glucose uptake.

[0037] In an alternative embodiment, the present invention provides a method of
20 identifying a compound that enhances glucose uptake comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein reduced ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound reduces fat deposition or enhances glucose uptake.

25 [0038] In an alternative embodiment, the present invention provides a method for identifying a compound that enhances glucose uptake comprising determining the level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein reduced phosphorylation of tyrosine residues in the Cbl protein in
30 the presence of the compound indicates that the compound enhances glucose uptake.

[0039] In an alternative embodiment, the invention provides a method of identifying a compound that reduces glucose uptake into liver, fat or muscle cells such as, for example, in the treatment of obesity, said method comprising: (a) administering a

compound that enhances glucose uptake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal and determining the glucose uptake into liver, fat or muscle cells; (b) administering a
5 compound to the animal and determining the glucose uptake into liver, fat or muscle cells of the animal, wherein a similar or reduced uptake at (b) compared to (a) indicates that the compound reduces glucose uptake into liver, fat or muscle cells.

[0040] In an alternative embodiment, the invention provides a method of identifying a
10 compound that reduces glucose uptake into liver, fat or muscle cells comprising: (a) administering a compound to a non-human animal expressing a functional Cbl protein and determining the glucose uptake into liver, fat or muscle cells of the animal; (b) determining the glucose uptake into liver, fat or muscle cells of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus
15 wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (c) comparing the glucose uptake into liver, fat or muscle cells of the animals at (a) and (b) wherein a comparable uptake between (a) and (b) indicates that the compound reduces glucose uptake into liver, fat or muscle cells.

20 [0041] In an alternative embodiment, the present invention provides a method of identifying a compound that reduces glucose uptake comprising determining the ubiquitin ligase activity of a Cbl protein in the presence and absence of the compound wherein enhanced ubiquitin ligase activity of the Cbl protein in the presence of the compound indicates that the compound reduces glucose uptake.

25

[0042] In an alternative embodiment, the present invention provides a method for identifying a compound that reduces glucose uptake comprising determining the level of tyrosine phosphorylation of a Cbl protein in the presence and absence of the compound wherein enhanced phosphorylation of tyrosine residues in the Cbl protein in
30 the presence of the compound indicates that the compound reduces glucose uptake.

[0043] A further aspect of the present invention provides methods for determining a modulator of the activity, formation or stability of a protein complex selected from the group consisting of: (i) a Cbl-APS complex; (ii) a Cbl-CAP complex; (iii) a Cbl-CAP-

flotillin complex; (iv) a Cbl- C3G complex; (v) a Cbl-CrkII complex; and (vi) a Cbl-C3G-CrkII complex.

[0044] In a further aspect, the invention provides methods relating to the treatment of animal or human subjects wherein a compound that modulates (i.e. enhances or reduces or prevents) Cbl expression or activity is administered to the animal. Such methods apply *mutatis mutandis* to the treatment of a wide range of conditions associated with Cbl function, such as, for example, hyperglycemia, hyperinsulinemia, obesity, adult-onset obesity, non-insulin-dependent diabetes mellitus, type II diabetes, glucose intolerance, hypertrophy or hyperplasia of the islets of Langerhans, or for cosmetic purposes, such as, for example, bodybuilding or weight management (i.e. weight loss or weight gain). It is to be understood that such applications of the invention also relate to the productivity of stock and farm animals (e.g. dairy and beef cattle, pigs, horses, sheep, etc) such as, for example, for modulating the amount of fat they deposit or their ratio of fat to muscle mass. In accordance with this aspect of the invention, an amount of a Cbl agonist or antagonist is administered to the animal or human subject effective to modulate the expression or activity of Cbl in the subject.

[0045] In another embodiment, the invention also provides a method of treating a feeding disorder characterized by reduced dietary intake or suppressed appetite in a subject said method comprising administering to the subject an amount of a Cbl antagonist effective to enhance the appetite or dietary intake of the subject. The method of the invention is particularly suited to the treatment of anorexia or bulimia.

[0046] In a related embodiment, the invention also provides a method of treating a feeding disorder characterized by reduced dietary intake or suppressed appetite in a subject said method comprising administering to the subject an amount of a compound that reduces expression of functional Cbl effective to enhance the appetite or dietary intake of the subject.

30

Brief description of the drawings

[0047] Figure 1 is a graphical representation showing the body weight in grams (ordinate) of male (circles) and female (triangles) wild type mice (open symbols), and Cbl-deficient male (circles) and female (triangles) mice that are homozygous for a

mutation in both alleles of the Cbl locus (filled symbols). Body weights of mice were determined every week from weaning to 16 weeks of age (x-axis). Data indicate that Cbl-deficient male mice have significantly higher body weight than their wild-type counterparts, throughout the time period tested. The weights of Cbl-deficient female mice are comparable to the weights of wild-type females.

[0048] Figure 2 is a graphical representation showing the dietary food intake in grams per day (ordinate) of male (circles) and female (triangles) wild type mice (open symbols), and Cbl-deficient male (circles) and female (triangles) mice that are homozygous for a mutation in both alleles of the Cbl locus (filled symbols). Food intake was determined every week from 5 weeks of age until 16 weeks of age (x-axis). Data indicate that Cbl-deficient male and female mice have significantly enhanced dietary intake (i.e. enhanced appetite) than their wild-type counterparts, throughout the time period tested. The dietary intake of Cbl-deficient female mice is also significantly higher than the dietary intake of wild-type males.

[0049] Figure 3 is a graphical representation showing the dietary food intake relative to body weight (ordinate) of male (circles) and female (triangles) wild type mice (open symbols), and Cbl-deficient male (circles) and female (triangles) mice that are homozygous for a mutation in both alleles of the Cbl locus (filled symbols). Food intake relative to body weight was determined every week from 5 weeks of age until 16 weeks of age (x-axis). Data indicate that Cbl-deficient male and female mice have significantly enhanced specific dietary intake (i.e. enhanced appetite as determined by food intake relative to body weight) than their wild-type counterparts, throughout the time period tested.

[0050] Figure 4 is a tabular representation showing the tissue weights (average \pm SEM) of wild type mice and Cbl-deficient mice that are homozygous for a mutation in both alleles of the Cbl locus (filled symbols). WAT, white adipose tissue; BAT, brown adipose tissue; QUAD, quadriceps muscle. Data indicate reduced adipose tissue weight for Cbl-deficient animals relative to wild type animals.

[0051] Figure 5 is a photographic representation showing adipocyte size in wild type mice (left) and Cbl-deficient mice that are homozygous for a mutation in both alleles of the Cbl locus (right).

- 5 [0052] Figure 6 is a tabular representation showing adipocyte diameter (μm) adipocyte volume (pl/cell) and lipid content (ng/cell) of male (Top panel) and female (Lower panel) wild type mice (WT), compared to male (Top panel) and female (Lower panel) Cbl-deficient mice that are homozygous for a mutation in both alleles of the Cbl locus (KO). Data indicate the mean values \pm SEM. Data indicate that for both males and
- 10 females, there is a significant reduction in adipocyte size and volume, and reduced lipid content of adipocytes in Cbl-deficient mice relative to wild-type mice.

Detailed description of the Preferred embodiments

- 15 [0053] One aspect of the present invention provides a method of identifying a compound that suppresses or reduces feeding behaviour, such as, for example, in the treatment of obesity, said method comprising: (a) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression
- 20 of functional Cbl in said animal; and (b) determining the feeding behaviour of the animal, wherein reduced appetite or dietary intake of the animal compared to the appetite or dietary intake of a Cbl-deficient animal to which the compound has not been administered indicates that the compound suppresses or reduces feeding behaviour.

25

- [0054] As used herein, the term "Cbl" shall be taken to mean any peptide, polypeptide, or protein having at least about 80% amino acid sequence identity to the amino acid sequence of a human or mouse c-Cbl polypeptide set forth in SEQ ID NO: 2 or 3. The term "Cbl" shall also be taken to include a peptide, polypeptide or protein having the
- 30 known biological activity of Cbl, or the known binding specificity of Cbl including c-Cbl. For the purposes of nomenclature, the amino acid sequences of the murine and human Cbl polypeptides are exemplified herein, as SEQ ID Nos: 2 and 3, respectively. Preferably, the percentage identity to SEQ ID NO: 2 or 3 is at least about 85%, more

preferably at least about 90%, even more preferably at least about 95% and still more preferably at least about 99%.

[0055] A "Cbl" protein generally comprises about 906 amino acid residues in length.

- 5 The full-length protein generally comprises a tyrosine kinase binding domain that binds the protein to phosphotyrosine residues, thereby coupling Cbl to growth factor receptor signalling. For example, the c-Cbl is phosphorylated on 3-4 tyrosine residues in response to growth factors, the phosphorylated residues representing binding domains for SH2 domain containing proteins such as PI 3' kinase and Crk II. The tyrosine
- 10 kinase binding domain generally will comprise a four helix bundle, an EF hand domain and an SH2 domain. Downstream of the tyrosine kinase binding domain is generally a C3HC4 RING finger domain having high sequence similarity to that found in ubiquitin ligase proteins. At the C terminal portion of the protein there is generally a proline rich region (PRR) which can bind SH3 domain-containing proteins (e.g. CAP). At the
- 15 extreme C terminus of Cbl there is generally located a leucine zipper domain (b-Zip) and a ubiquitin association domain that regulates homodimerization of Cbl.

- [0056] The various embodiments of the present invention directed to the identification of compounds that modulate feeding behaviour (e.g. in the treatment of obesity, anorexia or bulimia), fat deposition, metabolic rate, the ratio of lean muscle mass to
- 20 body fat, or glucose uptake (e.g. in the treatment of obesity or type II diabetes), can also be carried out by determining a physical property of the Cbl protein, or alternatively, a catalytic activity of the Cbl protein, that is modified during glucose uptake into fat cells or muscle cells.

25

- [0057] The ability of a compound to enhance feeding behaviour, reduce fat deposition, enhance metabolic rate, or enhance the ratio of lean muscle mass to body fat can thus be readily assayed by determining whether or not the compound reduces a Cbl activity that is disrupted in the Cbl-deficient mouse model. For example, reduced ubiquitin
- 30 ligase activity of Cbl protein, or reduced phosphorylation of tyrosine residues in the Cbl protein (e.g. linked to growth receptor-mediated signalling events such as by measuring cAMP-mediated phosphorylation), or reduced binding of Cbl to an SH3-containing protein, would indicate the ability of a compound to produce these effects. Standard assays for determining tyrosine phosphorylation of Cbl, receptor-mediated

signalling of tyrosine phosphorylation in Cbl protein, ubiquitin ligase activity of Cbl or binding of Cbl to SH3 domains, are known to the skilled artisan.

5 [0058] Similarly, the ability of a compound to reduce feeding behaviour, enhance fat deposition, reduce metabolic rate, or enhance the ratio of body fat to lean muscle mass can be assayed by determining whether or not the compound enhances ubiquitin ligase activity of Cbl protein, or enhances phosphorylation of tyrosine residues in the Cbl protein (e.g. linked to growth receptor-mediated signalling events such as by measuring cAMP-mediated phosphorylation), or enhances binding of Cbl to
10 an SH3-containing protein.

[0059] By "Cbl-deficient" is meant that insufficient functional Cbl protein is produced to facilitate the level of glucose uptake or glycogen synthesis or lipogenesis detected in a wild type animal that does not suffer from a disorder of glucose metabolism. Glucose
15 uptake, glycogen synthesis, or lipogenesis can readily be determined using known methods, such as, for example, by determining 2-deoxyglucose uptake into isolated liver, fat or muscle cells and/or glycogen synthesis and/or lipogenesis in the presence and absence of insulin as described by Lazar et al., J. Biol. Chem. 270: 20801-20807, 1995.

20

[0060] Preferably, the Cbl deficiency causes reduced Cbl expression at least in liver, fat or muscle cells of the genetically modified animal, more preferably in a tissue selected from the group consisting of adipose, skeletal muscle and cardiac muscle. Effects on Cbl expression in other cells or tissues, such as, for example, immune cells
25 or brain, are not to be excluded from the scope of the present invention.

[0061] In one embodiment, the genetic modification capable of producing the Cbl deficient phenotype is selected from the group consisting of a deletion, an insertion, a substitution and an inversion of nucleotides in an allele of the Cbl locus. In another
30 embodiment, the genetic modification is a deletion of a nucleotide sequence within two alleles of the Cbl locus, wherein the deletion results in an absence of expression of a functional or full-length Cbl protein by the animal. In a particularly preferred embodiment, the genetic modification comprises the deletion of a nucleotide sequence encoding a protein-encoding portion of the Cbl gene sufficient to prevent Cbl function,

or alternatively, the introduction of an in-frame stop codon at a location in the protein-encoding portion of the Cbl gene sufficient to prevent expression of functional Cbl by one or both Cbl-encoding alleles. Even more preferably, the genetic modification comprises the targeted disruption of one or two alleles at the Cbl locus produces a
5 truncated Cbl protein comprising the amino acid sequence set forth in SEQ ID NO: 1.

[0062] Preferably, the genetic modification is in both alleles of the Cbl locus (i.e. the animal is homozygous for the genetic modification).

10 [0063] The non-human animal is any mammal, such as, for example, a rodent, dog, pig, bovine, sheep, horse or goat. In one embodiment, the animal is a rodent selected from the group consisting of rabbit, rat, guinea pig and mouse. Conveniently, the animal is a mouse.

15 [0064] The Cbl-deficient "control" animal employed in this context can be any other animal that has reduced expression of Cbl expression and need not be isogenic to the animal on which the compound was tested (i.e. the "test" animal). Preferably, the control and test animals express similar levels of functional Cbl. More preferably, the control and test animals are isogenic. Preferably, the appetite or dietary intake of the
20 animal is modified to a level that is comparable to the appetite or dietary intake of a wild-type animal and does not completely suppress appetite or dietary intake if the animal.

[0065] By "feeding behaviour" is meant appetite or the amount of food consumption of
25 the animal in a particular time interval, or its dietary intake expressed in absolute terms or alternatively, as a proportion of total body mass. Dietary intake will also be generally determined relative to a particular time interval (e.g. per hour, per day, etc). In most non-human animals, dietary intake is the preferred measure.

30 [0066] The range of compounds contemplated herein for modulating feeding behaviour include peptides, including peptides derived from Cbl and capable of complementing the Cbl-deficiency; non-Cbl peptides, such as, for example Cbl peptidomimetics; small organic molecules, such as, for example derived from publicly available combinatorial

libraries; and nucleic acids, including nucleic acid encoding said peptide derived from Cbl or said non-Cbl peptide.

- [0067] In one embodiment, the subject method further comprises formulating the identified compound for administration to a non-human animal or a human. The formulations can be suitable for administration by injection by a subcutaneous, intravenous, intranasal, or intraperitoneal route. Alternatively, they can be suitable for oral administration in the form of feed additives, tablets, troches, etc.
- [0068] The compounds are conveniently formulated in a suitable excipient or diluent, such as, for example, an aqueous solvent, non-aqueous solvent, non-toxic excipient, such as a salt, preservative, buffer and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyl oleate. Aqueous solvents include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the formulation suitable for administration to the animal are adjusted according to routine skills in the art. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as

sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. For
5 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the
10 active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0069] Optionally, the formulation will also include a carrier, such as, for example, bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), ovalbumin, mouse
15 serum albumin, rabbit serum albumin and the like. Means for conjugating peptides to carrier proteins are also well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

20 [0070] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal.

[0071] Peptidyl compounds are conveniently made by standard peptide synthesis, such as the Merrifield method of synthesis (Merrifield, *J Am Chem Soc*, 85, 2149-
25 2154, 1963) and the myriad of available improvements on that technology (see e.g., *Synthetic Peptides: A User's Guide*, Grant, ed. (1992) W.H. Freeman & Co., New York, pp. 382; Jones (1994) *The Chemical Synthesis of Peptides*, Clarendon Press, Oxford, pp. 230.); Barany, G. and Merrifield, R.B. (1979) in *The Peptides* (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York; Wünsch, E.,
30 ed. (1974) *Synthese von Peptiden in Houben-Weyls Methoden der Organischen Chemie* (Müller, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart; Bodanszky, M. (1984) *Principles of Peptide Synthesis*, Springer-Verlag, Heidelberg; Bodanszky, M. & Bodanszky, A. (1984) *The Practice of Peptide Synthesis*, Springer-Verlag, Heidelberg; Bodanszky, M. (1985) *Int. J. Peptide Protein Res.* 25, 449-474.

[0072] Preferably, the peptide is synthesized on a solid phase support, such as, for example, a polystyrene gel bead comprising polystyrene cross-linked with divinylbenzene, preferably 1% (w.w) divinylbenzene, which is further swollen using lipophilic solvent, such as, for example dichloromethane or dimethylformamide (DMF). The polystyrene can be functionalized by addition of chloromethane or amino methyl groups. Alternatively, cross-linked and functionalized polydimethyl-acrylamide gel can be used once swollen and solvated using DMF or dipolar aprotic solvent. Other solid phase supports known to those skilled in the art can also be used for peptide synthesis, such as, for example, polyethylene glycol-derived bead produced by grafting polyethylene glycol to the surface of inert polystyrene beads. Preferred commercially available solid phase supports include PAL-PEG-PS, PAC-PEG-PS, KA, KR, or TGR (Applied Biosystems, CA 94404, USA).

[0073] For solid phase peptide synthesis, blocking groups that are stable to the repeated treatments necessary for removal of the amino blocking group of the growing peptide chain and for repeated amino acid couplings, are used for protecting the amino acid side-chains during synthesis and for masking undesired reactivity of the α -amino, carboxyl or side chain functional groups. Blocking groups (also called protecting groups or masking groups) thus protect the amino group of the amino acid having an activated carboxyl group that is involved in the coupling reaction, or protect the carboxyl group of the amino acid having an acylated amino group that is involved in the coupling reaction.

[0074] During synthesis, coupling occurs following removal of a blocking group without the disruption of a peptide bond, or any protecting group attached to another part of the peptide. Additionally, the peptide-resin anchorage that protects the C-terminus of the peptide is protected throughout the synthetic process until cleavage from the resin is required. Accordingly, by the judicious selection of orthogonally protected α -amino acids, amino acids are added at desired locations to a growing peptide whilst it is still attached to the resin.

[0075] Preferred amino blocking groups are easily removable but sufficiently stable to survive conditions for the coupling reaction and other manipulations, such as, for

example, modifications to the side-chain groups. In one embodiment, amino blocking groups are selected from the group consisting of: (i) a benzyloxycarbonyl group (Z or carbocenzoxy) that is removed easily by catalytic hydrogenation at room temperature and ordinary pressure, or using sodium in liquid ammonia and hydrobromic acid in acetic acid; (ii) a urethane derivative; (iii) a t-Butoxycarbonyl group (Boc) that is introduced using t-butoxycarbonyl azide or di-tert-butylidicarbonate and removed using mild acid such as, for example, trifluoroacetic acid (50% TFA in dichloromethane), or HCl in acetic acid/dioxane/ethylacetate; (iv) a 9-fluorenylmethyloxycarbonyl group (Fmoc), that is cleaved under mildly basic, non-hydrolytic conditions, such as, for example, using a primary or secondary amine (eg. 20% piperidine in dimethyl formamide); (v) a 2-(4-biphenyl) propyl(2)oxycarbonyl group (Bpoc); (vi) a 2-nitrophenylsulfenyl group (Nps); and (vii) a dithia-succionyl group (Dts). Boc is widely used to protect the N-terminus in Fmoc chemistry, or Fmoc is widely used to protect the N-terminus in Boc chemistry.

[0076] Side chain-protecting groups will vary for the functional side chains of the amino acids forming the peptide being synthesized. Side-chain protecting groups are generally based on the Bzl group or the tBu group. Amino acids having alcohols or carboxylic acids in the side-chain are protected as Bzl ethers, Bzl esters, cHex esters, tBu ethers, or tBu esters. Side-chain protection of Fmoc amino acids requires blocking groups that are ideally base stable and weak acid (TFA) labile. Many different protecting groups for peptide synthesis have been described (see *The Peptides*, Gross *et al.* eds., Vol. 3, Academic Press, New York, 1981). For example, the 4-methoxy-2,3,6-trimethylphenylsulfonyl (Nd- Mtr) group is useful for Arginine side-chain protection, however deprotection of Arg(Mtr) requires prolonged TFA treatment. A number of soft acid (TFA, thalium (III) trifluoroacetate/TFA) labile groups, or TFA stable but thalium (III) trifluoroacetate/TFA labile groups, or soft acid stable groups are used to protect Cystine.

[0077] The two most widely used protection strategies are the Boc/Bzl- and the Fmoc/tBu-strategies. In Boc/Bzl, Boc is used for amino protection and the side-chains of the various amino acids are protected using Bzl- or cHex-based protecting groups. A Boc group is stable under catalytic hydrogenation conditions and is used orthogonally along with a Z group for protection of many side chain groups. In

Fmoc/tBu, Fmoc is used for amino protection and the side-chains are protected with tBu-based protecting groups.

[0078] Alternatively, the peptidyl compound is produced by the recombinant
5 expression of nucleic acid encoding the amino acid sequence of said peptide. Random
peptide-encoding libraries are particularly preferred for such purposes, because they
provide a wide range of different compounds to test. Alternatively, naturally-occurring
nucleic acids can be screened. According to this embodiment, nucleic acid encoding
the peptidyl compound is produced by standard oligonucleotide synthesis or derived
10 from a natural source and cloned into a suitable expression vector in operable
connection with a promoter or other regulatory sequence capable of regulating
expression in a cell-free system or cellular system..

[0079] Oligonucleotides are preferably synthesized with linker or adaptor sequences at
15 the 5'- and 3'-ends to facilitate subsequent cloning into a suitable vector system using
standard techniques.

[0080] Placing a nucleic acid molecule under the regulatory control of, i.e., "in operable
connection with", a promoter sequence means positioning said molecule such that
20 expression is controlled by the promoter sequence, generally by positioning the
promoter 5' (upstream) of the peptide-encoding sequence.

[0081] The prerequisite for producing intact peptides in bacteria such as *E. coli* is the
use of a strong promoter with an effective ribosome binding site. Typical promoters
25 suitable for expression in bacterial cells such as *E. coli* include, but are not limited to,
the *lacZ* promoter, temperature-sensitive λ_L or λ_R promoters, T7 promoter or the IPTG-
inducible *tac* promoter. A number of other vector systems for expressing the nucleic
acid molecule of the invention in *E. coli* are well-known in the art and are described, for
example, in Ausubel *et al* (*In: Current Protocols in Molecular Biology*, Wiley
30 Interscience, ISBN 047150338, 1987) or Sambrook *et al* (*In: Molecular cloning, A
laboratory manual, second edition, Cold Spring Harbor Laboratory, Cold Spring
Harbor, N.Y., 1989*). Numerous plasmids with suitable promoter sequences for
expression in bacteria and efficient ribosome binding sites have been described, such
as for example, pKC30 (λ_L : Shimatake and Rosenberg, *Nature* 292, 128, 1981);

pKK173-3 (*lac*: Amann and Brosius, *Gene* 40, 183, 1985), pET-3 (T7: Studier and Moffat, *J. Mol. Biol.* 189, 113, 1986); the pBAD/TOPO or pBAD/Thio-TOPO series of vectors containing an arabinose-inducible promoter (Invitrogen, Carlsbad, CA), the latter of which is designed to also produce fusion proteins with thioredoxin to enhance
 5 solubility of the expressed protein; the pFLEX series of expression vectors (Pfizer Inc., CT, USA); or the pQE series of expression vectors (Qiagen, CA), amongst others.

[0082] Typical promoters suitable for expression in viruses of eukaryotic cells and eukaryotic cells include the SV40 late promoter, SV40 early promoter and
 10 cytomegalovirus (CMV) promoter, CMV IE (cytomegalovirus immediate early) promoter amongst others. Preferred vectors for expression in mammalian cells (eg. 293, COS, CHO, 10T cells, 293T cells) include, but are not limited to, the pcDNA vector suite supplied by Invitrogen, in particular pcDNA 3.1 myc-His-tag comprising the CMV promoter and encoding a C-terminal 6xHis and MYC tag; and the retrovirus
 15 vector pSR α tkneo (Muller *et al.*, *Mol. Cell. Biol.*, 11, 1785, 1991). The vector pcDNA 3.1 myc-His (Invitrogen) is particularly preferred for expressing peptides in a secreted form in 293T cells, wherein the expressed peptide or protein can be purified free of conspecific proteins, using standard affinity techniques that employ a Nickel column to bind the protein via the His tag.

20

[0083] A wide range of additional host/vector systems suitable for expressing peptides are available publicly, and described, for example, in Sambrook *et al* (*In: Molecular cloning, A laboratory manual, second edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989*).

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[0084] Means for introducing the nucleic acid or a gene construct comprising same into a cell for expression are well-known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into animal cells include microinjection, transfection
 30 mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

[0085] Techniques for synthesizing small organic compounds will vary considerably depending upon the compound, however such methods will be well known to those skilled in the art. In one embodiment, informatics is used to select suitable chemical building blocks from known compounds, for producing a combinatorial library. For example, QSAR(Quantitative Structure Activity Relationship) modelling approach uses linear regressions or regression trees of compound structures to determine suitability. The software of the Chemical Computing Group, Inc.(Montreal, Canada) uses high-throughput screening experimental data on active as well as inactive compounds, to create a probabilistic QSAR model, which is subsequently used to select lead compounds. The Binary QSAR method is based upon three characteristic properties of compounds that form a "descriptor" of the likelihood that a particular compound will or will not perform a required function: partial charge, molar refractivity (bonding interactions), and logP (lipophilicity of molecule). Each atom has a surface area in the molecule and it has these three properties associated with it. All atoms of a compound having a partial charge in a certain range are determined and the surface areas (Van der Waals Surface Area descriptor) are summed. The binary QSAR models are then used to make activity models or ADMET models, which are used to build a combinatorial library. Accordingly, information from known appetite suppressants and non-suppressants, including lead compounds identified in initial screens, can be used to expand the list of compounds being screened to thereby identify highly active compounds.

[0086] Another embodiment of the invention provides a method of identifying a compound that enhances feeding behaviour, such as, for example, in the treatment of anorexia or bulimia, said method comprising: (a) administering a compound that suppresses appetite or dietary intake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal and determining the feeding behaviour of the animal; (b) administering a compound to the animal and determining the feeding behaviour of the animal, wherein enhanced appetite or dietary intake at (b) compared to (a) indicates that the compound enhances feeding behaviour.

[0087] Naturally, the compound administered at (b) will be different to the compound at (a).

[0088] Preferably, the compound that suppresses appetite or dietary intake acts via a Cbl-mediated mechanism, which can be verified using the genetically modified Cbl-deficient animal, such as by determining the ability of the compound to suppress appetite or dietary intake of Cbl-deficient mice according to a method described herein. In this case, step (a) *supra* of administering a compound that suppresses appetite or dietary intake to the genetically modified non-human animal will comprise:

5 (i) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (ii) determining the feeding behaviour of the animal, wherein reduced appetite or dietary intake of the animal compared to a Cbl-deficient animal to which the compound has not been

10 administered indicates that the compound suppresses appetite or dietary intake.

15

[0089] In an alternative embodiment, the invention provides a method of identifying a compound that modulates feeding behaviour, such as, for example, in the treatment of anorexia or bulimia, said method comprising: (a) administering a compound to a non-

20 human animal expressing a functional Cbl protein and determining the feeding behaviour of the animal; (b) determining the feeding behaviour of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (c) comparing the feeding behaviour of the animals

25 at (a) and (b) wherein a comparable feeding behaviour between (a) and (b) indicates that the compound modulates feeding behaviour.

[0090] In one embodiment, the subject method further comprises formulating the identified compound for administration to a non-human animal or a human as

30 described *supra*.

[0091] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal.

[0092] The range of compounds contemplated herein include Cbl inhibitory compounds or antagonists of a biological function of Cbl. In one embodiment, the compound is selected from the group consisting of: a peptide, including a peptide derived from Cbl and capable of inhibiting, reducing or repressing a Cbl function, including binding to a protein selected from the group consisting of CAP, Crkl and C3G; a Cbl dominant-negative mutant; a non-Cbl peptide inhibitors of Cbl; an antibody or antibody fragment which binds to Cbl and inhibits a Cbl function; a small organic molecule, and nucleic acid, including nucleic acid encoding said peptide derived from Cbl or said non-Cbl peptide inhibitor, an antisense nucleic acid directed against Cbl-encoding mRNA, or an anti-Cbl ribozyme, or a small interfering RNA (RNAi) that targets Cbl gene expression.

[0093] The term "antisense nucleic acid" shall be taken to mean DNA or RNA molecule that is complementary to at least a portion of a specific mRNA molecule (Weintraub, Scientific American 262:40, 1990) and capable of interfering with a post-transcriptional event such as mRNA translation. Antisense oligomers complementary to at least about 15 contiguous nucleotides of Cbl-encoding mRNA are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target Cbl-producing cell. The use of antisense methods is well known in the art (Marcus-Sakura, Anal. Biochem. 172: 289, 1988). Preferred antisense nucleic acid will comprise a nucleotide sequence that is complementary to at least 15 contiguous nucleotides of a sequence encoding the amino acid sequence set forth in SEQ ID NO: 2 or 3.

[0094] As used herein, a "ribozyme" is a nucleic acid molecule having nuclease activity for a specific nucleic acid sequence. A ribozyme specific for Cbl-encoding mRNA, for example, binds to and cleaves specific regions of the mRNA, thereby rendering it untranslatable. To achieve specificity, preferred ribozymes will comprise a nucleotide sequence that is complementary to at least about 12-15 contiguous nucleotides of a sequence encoding the amino acid sequence set forth in SEQ ID NO: 2 or 3.

[0095] As used herein, the terms "small interfering RNA", and "RNAi" refer to homologous double stranded RNA (dsRNA) that specifically targets a gene product, thereby resulting in a null or hypomorphic phenotype. Specifically, the dsRNA

comprises two short nucleotide sequences derived from the target RNA encoding Cbl and having self-complementarity such that they can anneal, and interfere with expression of a target gene, presumably at the post-transcriptional level. RNAi molecules are described by Fire et al., Nature 391, 806-811, 1998, and reviewed by
5 Sharp, Genes & Development, 13, 139-141, 1999).

[0096] The term "dominant-negative mutant" refers to a Cbl polypeptide that has been mutated from its natural state and that interacts with a protein that Cbl normally interacts with thereby preventing endogenous native Cbl from forming the interaction.
10 Preferred dominant negative mutants will lack that portion of Cbl that interacts with a protein selected from the group consisting of CAP, CrklI and C3G.

[0097] The "antibodies" contemplated herein are immunoreactive with Cbl polypeptides or functional fragments thereof. Antibodies which consist essentially of
15 pooled monoclonal antibodies with different epitope specificities, as well as distinct monoclonal antibody preparations are contemplated. Monoclonal antibodies are produced from fragments of the Cbl protein that comprise one or more B cell epitopes by methods well known to those skilled in the art (Kohler et al, Nature 256:495, 1975). The term "antibody" as used herein includes intact molecules as well as fragments
20 thereof, such as Fab and F(ab')₂, Fv and single chain antibody fragments capable of binding an epitopic determinant of Cbl.

[0098] An "Fab fragment" consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule
25 with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

[0099] An "Fab' fragment" of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of
30 an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

[0100] An "F(ab')₂ fragment" of an antibody consists of a dimer of two Fab' fragments held together by two disulfide bonds, and is obtained by treating a whole antibody

molecule with the enzyme pepsin, without subsequent reduction. A (Fab').sub.2 fragment.

5 [0101] An "Fv fragment" is a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

10 [0102] A "single chain antibody" (SCA) is a genetically engineered single chain molecule containing the variable region of a light chain and the variable region of a heavy chain, linked by a suitable, flexible polypeptide linker.

15 [0103] In one embodiment, peptidyl Cbl inhibitors are chemically or recombinantly synthesized as oligopeptides (approximately 10-25 amino acids in length) spanning the Cbl sequence (SEQ ID NO: 2 or 3). Alternatively, Cbl fragments are produced by digestion of native or recombinantly produced Cbl by, for example, using a protease, e.g., trypsin, thermolysin, chymotrypsin, or pepsin. Computer analysis (using commercially available software, e.g. MacVector, Omega, PCGene, Molecular Simulation, Inc.) is used to identify proteolytic cleavage sites. The proteolytic or synthetic fragments can comprise as many amino acid residues as are necessary to 20 partially or completely inhibit Cbl function. Preferred fragments will comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length.

25 [0104] In one embodiment, peptides are selected which contain a sufficient number of B cell epitopes to elicit antibodies when administered to a mammal. Such peptides can be identified by immunizing a mammal with the peptide, either alone or combined with or linked to an adjuvant (e.g., a hapten), and testing sera from the immunized animal for anti-Cbl antibodies. Preferred peptides generate anti-Cbl antibodies which inhibit a Cbl function.

30

[0105] Preferred peptidyl Cbl inhibitors will also not comprise a sufficient number of T cell epitopes to induce T-cell mediated (e.g., cytokine) responses when determined using any of a number of well known techniques, such as epitope prediction using algorithms (see e.g., Rothbard and Taylor EMBO J. 7: 93-100, 1988; Berzofsky, Philos

Trans R. Soc. Lond. 323: 535-544, 1989; Rothbard, 1st Forum in Virology, Annals of the Pasteur Institute, pp 518-526, Dec. 1986; Rothbard and Taylor, Embo, 7: 93-100, 1988; EP 0 304 279; and Margalit et al., J. Immunol., 138: 2213-2229, 1987); or screening of peptide inhibitors for human T cell stimulating activity or T cell proliferation assays (e.g. Proc. Natl. Acad. Sci USA, 86:1333, 1989).

[0106] Other preferred peptide inhibitors of Cbl are located on the surface of the Cbl proteins, e.g., hydrophilic regions, as well as regions with high antigenicity or fragments with high surface probability scores can be identified using computer analysis programs well known to those of skill in the art (Hopp and Wood, (1983), Mol.Immunol., 20, 483-9, Kyte and Doolittle, (1982), J. Mol. Biol., 157, 105-32, Corrigan and Huang, (1982), Comput. Programs Biomed, 3, 163-8).

[0107] Anti-Cbl antibodies or antibody fragments are generated using the entire Cbl polypeptide or an immunogenic fragment thereof (alone or linked to a suitable carrier or hapten) to immunize a subject (e.g., a mammal including, but not limited to a rabbit, goat, mouse or other mammal). For example, the methods described in U.S. Pat. Nos. 5,422,110; 5,837,268; 5,708,155; 5,723,129; and 5,849,531, can be used and are incorporated herein by reference. In a preferred embodiment, the mammal being immunized does not contain endogenous Cbl (e.g., a Cbl-deficient genetically modified animal). The immunogenic preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic proteolytic or synthetic Cbl peptide preparation induces a polyclonal anti-Cbl antibody response. The anti-Cbl antibody titer in the immunized subject is generally monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized Cbl. Subsequently, the sera from the immunized subjects can be tested for Cbl inhibitory activity.

[0108] Alternatively, is also possible to immunize the subject with nucleic acid expressing Cbl using DNA immunization technology, such as that disclosed in U.S. Pat. No. 5,795,872 to Ricigliano et al., or in U.S. Pat. No. 5,643,578 to Robinson et al.

[0109] The antibody molecules directed against Cbl can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-Cbl antibody titers are highest, antibody-producing cells can be
5 obtained from the subject and used to prepare e.g., monoclonal antibodies by standard techniques, such as the hybridoma technique originally described in the following disclosures: Kohler and Milstein *Nature* 256:495-497, 1975; Brown et al. *J. Immunol.* 127:53946, 1981; Brown et al. *J. Biol Chem.* 255: 4980-4983, 1980; Yeh et al. *Proc. Natl. Acad. Sci. USA* 76:2927-2931, 1976; Yeh et al. *Int. J. Cancer* 29: 269-
10 275, 1982; Kozbor et al. *Immunol Today* 4:72, 1983; Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985. The technology for producing monoclonal antibody hybridomas is well known in the art. Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a Cbl peptide immunogen as described above, and
15 the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds Cbl.

[0110] Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-Cbl
20 monoclonal antibody (see, e.g., G. Galfre et al., *Nature* 266: 550-552, 1970). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse
25 immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-
30 Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed).

Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind Cbl, e.g., using a standard ELISA assay. The antibodies can then be tested for Cbl inhibitory activity.

- 5 [0111] A further aspect of the present invention provides a method of identifying a compound that enhances fat deposition or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate, such as, for example, in the treatment of hypolipidemia (e.g. induced by HIV antiviral or anti-cancer therapeutic), said method comprising: (a) administering a compound to a genetically modified non-
10 human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (b) determining the fat content of the animal, wherein enhanced fat content of the animal compared to the fat content of a Cbl-deficient animal to which the compound has not been administered indicates that the compound enhances fat
15 deposition or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate.

[0112] By "fat deposition" is meant the amount of fat deposited or the rate at which fat is deposited in liver, fat or muscle cells. Standard means known to the skilled artisan
20 are used to determine fat deposition. "Lean muscle mass" means muscle tissue that is substantially fat-free. The "ratio of body fat to muscle mass" means the relative amount of total body fat to total non-fat tissue. "Metabolic rate" means the ability of a subject to utilize dietary intake for immediate energy needs, rather than store such dietary intake as body fat.

25

[0113] The genetically modified animal and the Cbl-deficient animal are described *supra*.

[0114] The range of compounds contemplated herein for enhancing fat deposition or
30 reducing lean muscle mass or enhancing the ratio of body fat to muscle or reducing metabolic rate include peptides, including peptides derived from Cbl and capable of complementing the Cbl-deficiency; non-Cbl peptides, such as, for example Cbl peptidomimetics; small organic molecules, such as, for example derived from publicly available combinatorial libraries; and nucleic acids, including nucleic acid encoding

said peptide derived from Cbl or said non-Cbl peptide. Such compounds are described *supra*.

[0115] In one embodiment, the subject method further comprises formulating the
5 identified compound for administration to a non-human animal or a human. Such formulations are described *supra*.

[0116] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal. Such
10 methods are described *supra*.

[0117] In an alternative embodiment, the invention provides a method of identifying a compound that reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate, such as, for example, in the
15 treatment of obesity, said method comprising: (a) administering a compound that enhances fat deposition or glucose uptake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal and determining the fat content of the animal; (b) administering a compound to the animal
20 and determining the fat content of the animal, wherein a similar or reduced fat content at (b) compared to (a) indicates that the compound reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate.

25 [0118] Preferably, the animal is maintained on a diet comprising high glycemic index food, such as, for example, carrots or food supplemented with sucrose.

[0119] Preferably, the compound that enhances fat deposition, or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate acts via a
30 Cbl-mediated mechanism, which can be verified using the genetically modified Cbl-deficient animal, such as by determining the ability of the compound to enhance fat content of Cbl-deficient mice according to a method described herein. In this case, step (a) *supra* of administering a compound that enhances fat deposition or glucose uptake comprises (i) administering a compound to a genetically modified non-human

animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (ii) determining the fat content of the animal, wherein enhanced fat content of the animal compared to a Cbl-deficient animal to which the compound has not been administered indicates that the compound enhances fat deposition, or reduces lean muscle mass or enhances the ratio of body fat to muscle or reduces metabolic rate.

[0120] In an alternative embodiment, the invention provides a method of identifying a compound that reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate comprising: (a) administering a compound to a non-human animal expressing a functional Cbl protein and determining the fat content of the animal; (b) determining the fat content of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (c) comparing the fat contents of the animals at (a) and (b) wherein a comparable fat content between (a) and (b) indicates that the compound reduces fat deposition or enhances lean muscle mass or reduces the ratio of body fat to muscle or enhances metabolic rate.

[0121] Preferably, the animals are maintained on similar or identical diets, more preferably, on diets comprising high glycemic index food.

[0122] The range of compounds contemplated herein include Cbl inhibitory compounds or antagonists of a biological function of Cbl. In one embodiment, the compound is selected from the group consisting of: a peptide, including a peptide derived from Cbl and capable of inhibiting, reducing or repressing a Cbl function, including binding to a protein selected from the group consisting of CAP, Crkl and C3G; a Cbl dominant-negative mutant; a non-Cbl peptide inhibitors of Cbl; an antibody or antibody fragment which binds to Cbl and inhibits a Cbl function; a small organic molecule, and nucleic acid, including nucleic acid encoding said peptide derived from Cbl or said non-Cbl peptide inhibitor, an antisense nucleic acid directed against Cbl-encoding mRNA, an anti-Cbl ribozyme, RNAi or siRNA.

[0123] In one embodiment, the subject method further comprises formulating the identified compound for administration to a non-human animal or a human. Such formulations are described *supra*.

- 5 [0124] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal. Such methods are described *supra*.

[0125] A further aspect of the present invention provides a method of identifying a
10 compound that enhances glucose uptake such as, for example, in the treatment of hypolipidemia (e.g. induced by HIV antiviral or anti-cancer therapeutic), said method comprising: (a) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal; and (b)
15 determining the glucose uptake into liver, fat or muscle cells of the animal, wherein enhanced uptake compared to the glucose uptake into liver, fat or muscle cells of a Cbl-deficient animal to which the compound has not been administered indicates that the compound enhances glucose uptake.

20 [0126] Means for determining glucose uptake are well known in the art. Preferably, the process is performed *ex vivo* using liver, fat or muscle cells that have been previously isolated from the animal. In one embodiment, the glucose uptake is basal glucose uptake (i.e. glucose uptake measured in the absence of exogenously administered insulin). In another embodiment, the glucose uptake is insulin-mediated
25 glucose uptake (i.e. glucose uptake measured following administration of insulin). All embodiments of the invention described herein apply *mutatis mutandis* to both basal glucose uptake and insulin-mediated glucose uptake unless otherwise stated.

[0127] The genetically modified animal and the Cbl-deficient animal are described
30 *supra*.

[0128] The range of compounds contemplated herein for enhancing glucose uptake include peptides, including peptides derived from Cbl and capable of complementing the Cbl-deficiency; non-Cbl peptides, such as, for example Cbl peptidomimetics; small

organic molecules, such as, for example derived from publicly available combinatorial libraries; and nucleic acids, including nucleic acid encoding said peptide derived from Cbl or said non-Cbl peptide. Such compounds are described *supra*.

- 5 [0129] In one embodiment, the subject method further comprises formulating the identified compound for administration to a non-human animal or a human. Such formulations are described *supra*.

- 10 [0130] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal. Such methods are described *supra*.

- 15 [0131] In an alternative embodiment, the invention provides a method of identifying a compound that reduces glucose uptake into liver, fat or muscle cells, such as, for example, in the treatment of obesity, said method comprising: (a) administering a compound that enhances glucose uptake to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in said animal and determining the glucose uptake into liver, fat or muscle cells; (b) administering a compound to the animal and determining the glucose uptake into liver, fat or muscle cells of the animal, wherein a similar or reduced uptake at (b) compared to (a) indicates that the compound reduces glucose uptake into liver, fat or muscle cells.

- 25 [0132] Preferably, the animal is maintained on a diet comprising high glycemic index food, such as, for example, carrots or food supplemented with sucrose.

- 30 [0133] Preferably, the compound that enhances glucose uptake into liver, fat or muscle cells acts via a Cbl-mediated mechanism, which can be verified using the genetically modified Cbl-deficient animal, such as by determining the ability of the compound to enhance glucose uptake into liver, fat or muscle cells of Cbl-deficient mice according to a method described herein. In this case, step (a) *supra* of administering a compound that enhances glucose uptake into liver, fat or muscle cells comprises (i) administering a compound to a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification

reduces or prevents expression of functional Cbl in said animal; and (ii) determining the glucose uptake into liver, fat or muscle cells of the animal, wherein enhanced glucose uptake into liver, fat or muscle cells of the animal compared to a Cbl-deficient animal to which the compound has not been administered indicates that the compound
5 enhances glucose uptake into liver, fat or muscle cells.

[0134] In an alternative embodiment, the invention provides a method of identifying a compound that reduces glucose uptake into liver, fat or muscle cells comprising: (a) administering a compound to a non-human animal expressing a functional Cbl protein
10 and determining the glucose uptake into liver, fat or muscle cells of the animal; (b) determining the glucose uptake into liver, fat or muscle cells of a genetically modified non-human animal comprising a genetic modification within an allele of its Cbl locus wherein said genetic modification reduces or prevents expression of functional Cbl in
15 said animal; and (c) comparing the glucose uptake into liver, fat or muscle cells of the animals at (a) and (b) wherein a comparable uptake between (a) and (b) indicates that the compound reduces glucose uptake into liver, fat or muscle cells.

[0135] Preferably, the animals are maintained on similar or identical diets, more preferably, on diets comprising high glycemic index food.
20

[0136] The range of compounds contemplated herein include Cbl inhibitory compounds or antagonists of a biological function of Cbl. In one embodiment, the compound is selected from the group consisting of: a peptide, including a peptide derived from Cbl and capable of inhibiting, reducing or repressing a Cbl function,
25 including binding to a protein selected from the group consisting of CAP, Crkl and C3G; a Cbl dominant-negative mutant; a non-Cbl peptide inhibitors of Cbl; an antibody or antibody fragment which binds to Cbl and inhibits a Cbl function; a small organic molecule, and nucleic acid, including nucleic acid encoding said peptide derived from Cbl or said non-Cbl peptide inhibitor, an antisense nucleic acid directed against Cbl-
30 encoding mRNA, or an anti-Cbl ribozyme.

[0137] In one embodiment, the subject method further comprises formulating the identified compound for administration to a non-human animal or a human. Such formulations are described *supra*.

[0138] In another embodiment, the subject method further comprises producing or synthesizing the compound that is tested on the genetically modified animal. Such methods are described *supra*.

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[0139] A further aspect of the present invention provides methods for determining a modulator of the activity, formation or stability of a protein complex selected from the group consisting of: (i) a Cbl-APS complex; (ii) a Cbl-CAP complex; (iii) a Cbl-CAP-flotillin complex; (iv) a Cbl- C3G complex; (v) a Cbl-CrkII complex; and (vi) a Cbl-C3G-
10 CrkII complex.

[0140] In their general form, the methods of the present invention comprise determining the association or dissociation of the protein complex, or the structure of the complex, in the presence and absence of a candidate compound or a candidate
15 antibody. In accordance with the embodiment described herein, a modified association, dissociation, or structure, of the protein complex in the presence of a candidate compound or a candidate antibody indicates that the candidate is a modulator of the protein complex.

[0141] The association, dissociation, or structure of the complex may be determined by direct means, such as, for example, by determining real time association or dissociation constants in the presence and absence of the candidate, or modified binding of an antibody that recognizes a conformational epitope of the complex. Biosensors used essentially as described herein above, in the presence or absence of
20 the candidate compound or antibody, are particularly suited to such applications.
25

[0142] Alternatively, the association, dissociation, or structure of the complex may be determined by indirect means, such as, for example, using a protein recruitment system, n-hybrid screen, reverse n-hybrid screen, plate agar diffusion assay, ELISA,
30 or other well known assay format for detecting protein-protein interactions. Such indirect means generally use a reporter system to detect formation or dissociation of the protein complex.

[0143] Standard solid-phase ELISA assay formats are particularly useful for identifying antagonists of the protein-protein interaction. In accordance with this embodiment, one of the binding partners (e.g. APS or CAP or Crkl or C3G or a portion thereof) is immobilized on a solid matrix, such as, for example an array of polymeric pins or a glass support. Conveniently, the immobilized binding partner is a fusion polypeptide comprising Glutathione-S-transferase (GST; e.g. a CAP-GST fusion), wherein the GST moiety facilitates immobilization of the protein to the solid phase support. The second binding partner (e.g. Cbl) in solution is brought into physical relation with the immobilized protein to form a protein complex, which complex is detected using antibodies directed against the second binding partner. The antibodies are generally labelled with fluorescent molecules or conjugated to an enzyme (e.g. horseradish peroxidase), or alternatively, a second labelled antibody can be used that binds to the first antibody. Conveniently, the second binding partner is expressed as a fusion polypeptide with a FLAG or oligo-histidine peptide tag, or other suitable immunogenic peptide, wherein antibodies against the peptide tag are used to detect the binding partner. Alternatively, oligo-HIS tagged protein complexes can be detected by their binding to nickel-NTA resin (Qiagen), or FLAG-labeled protein complexes detected by their binding to FLAG M2 Affinity Gel (Kodak). It will be apparent to the skilled person that the assay format described herein is amenable to high throughput screening of samples, such as, for example, using a microarray of bound peptides or fusion proteins.

[0144] A two-hybrid assay is described in US Patent No. 6,316,223 to Payan *et al.*, incorporated herein by reference. The basic mechanism described by Payan *et al.* is similar to the yeast two hybrid system. In the two-hybrid system, the binding partners are expressed as two distinct fusion proteins in a mammalian host cell. In adapting the standard two-hybrid screen to the present purpose, a first fusion protein consists of a DNA binding domain which is fused to one of the binding partners, and a second fusion protein consists of a transcriptional activation domain fused to the other binding partner. The DNA binding domain binds to an operator sequence which controls expression of one or more reporter genes. The transcriptional activation domain is recruited to the promoter through the functional interaction between binding partners. Subsequently, the transcriptional activation domain interacts with the basal transcription machinery of the cell, thereby activating expression of the reporter

gene(s), the expression of which can be determined. Candidate bioactive agents that modulate the protein-protein interaction between the binding partners are identified by their ability to modulate transcription of the reporter gene(s) when incubated with the host cell. Antagonists will prevent or reduce reporter gene expression, while agonists will enhance reporter gene expression. In the case of small molecule modulators, these are added directly to the cell medium and reporter gene expression determined. On the other hand, peptide modulators are expressible from nucleic acid that is transfected into the host cell and reporter gene expression determined. In fact, whole peptide libraries can be screened in transfected cells.

10 [0145] Alternatively, reverse two hybrid screens, such as, for example, described by Vidal *et al.*, *Proc. Natl Acad. Sci USA* 93, 10315-10320, 1996, may be employed to identify antagonist molecules. Reverse hybrid screens differ from forward screens *supra* in so far as they employ a counter-selectable reporter gene, such as for example, *CYH2* or *LYS2*, to select against the protein-protein interaction. Cell survival or growth is reduced or prevented in the presence of a non-toxic substrate of the counter-selectable reporter gene product, which is converted by said gene product to a toxic compound. Accordingly, cells in which the protein-protein interaction of the invention does not occur, such as in the presence of an antagonist of said interaction, survive in the presence of the substrate, because it will not be converted to the toxic product. For example, a portion/fragment of Cbl that binds to APS or CAP or CrklI or C3G is expressed as a DNA binding domain fusion, such as with the DNA binding domain of GAL4; and the portion of APS or CAP or CrklI or C3G that binds Cbl is expressed as an appropriate transcription activation domain fusion polypeptide (e.g. with the GAL4 transcription activation domain). The fusion polypeptides are expressed in yeast in operable connection with the *URA3* counter-selectable reporter gene, wherein expression of *URA3* requires a physical relation between the GAL4 DNA binding domain and transcriptional activation domain. This physical relation is achieved, for example, by placing reporter gene expression under the control of a promoter comprising nucleotide sequences to which GAL4 binds. Cells in which the reporter gene is expressed do not grow in the presence of uracil and 5-fluororotic acid (5-FOA), because the 5-FOA is converted to a toxic compound. Candidate peptide inhibitor(s) are expressed in libraries of such cells, wherein cells that grow in the presence of uracil and 5-FOA are retained for further analysis, such as, for example,

analysis of the nucleic acid encoding the candidate peptide inhibitor(s). Small molecules that antagonize the interaction are determined by incubating the cells in the presence of the small molecules and selecting cells that grow or survive of cells in the presence of uracil and 5-FOA.

5

[0146] Alternatively, a protein recruitment system, such as that described in U.S. Patent No. 5, 776, 689 to Karin *et al.*, is used. In a standard protein recruitment system, a protein-protein interaction is detected in a cell by the recruitment of an effector protein, which is not a transcription factor, to a specific cell compartment.

10 Upon translocation of the effector protein to the cell compartment, the effector protein activates a reporter molecule present in that compartment, wherein activation of the reporter molecule is detectable, for example, by cell viability, indicating the presence of a protein-protein interaction.

15 [0147] More specifically, the components of a protein recruitment system include a first expressible nucleic acid encoding a first fusion protein comprising the effector protein and one of the binding partners (e.g. APS or CAP or CrkII or C3G or a portion thereof), and a second expressible nucleic acid molecule encoding a second fusion protein comprising a cell compartment localization domain and the other binding partner (e.g.
20 Cbl or a portion thereof). A cell line or cell strain in which the activity of an endogenous effector protein is defective or absent (e.g. a yeast cell or other non-mammalian cell), is also required, so that, in the absence of the protein-protein interaction, the reporter molecule is not expressed.

25 [0148] A complex is formed between the fusion polypeptides as a consequence of the interaction between the binding partners, thereby directing translocation of the complex to the appropriate cell compartment mediated by the cell compartment localization domain (e.g. plasma membrane localization domain, nuclear localization domain, mitochondrial membrane localization domain, and the like), where the effector
30 protein then activates the reporter molecule. Such a protein recruitment system can be practiced in essentially any type of cell, including, for example, mammalian, avian, insect and bacterial cells, and using various effector protein/reporter molecule systems.

[0149] For example, a yeast cell based assay is performed, in which the interaction between Cbl and one or more of its binding partners results in the recruitment of a guanine nucleotide exchange factor (GEF or C3G) to the plasma membrane, wherein GEF or C3G activates a reporter molecule, such as Ras, thereby resulting in the survival of cells that otherwise would not survive under the particular cell culture conditions. Suitable cells for this purpose include, for example, *Saccharomyces cerevisiae* cdc25-2 cells, which grow at 36°C only when a functional GEF is expressed therein, Petitjean *et al.*, *Genetics* 124, 797-806, 1990) Translocation of the GEF to the plasma membrane is facilitated by a plasma membrane localization domain. Activation of Ras is detected, for example, by measuring cyclic AMP levels in the cells using commercially available assay kits and/or reagents. To detect antagonists of the protein-protein interaction of the present invention, duplicate incubations are carried out in the presence and absence of a test compound, or in the presence or absence of expression of a candidate antagonist peptide in the cell. Reduced survival or growth of cells in the presence of a candidate compound or candidate peptide indicates that the peptide or compound is an antagonist of the interaction between Cbl and one or more of its binding partners.

[0150] A "reverse" protein recruitment system is also contemplated, wherein modified survival or modified growth of the cells is contingent on the disruption of the protein-protein interaction by the candidate compound or candidate peptide. For example, NIH 3T3 cells that constitutively express activated Ras in the presence of GEF can be used, wherein the absence of cell transformation is indicative of disruption of the protein complex by a candidate compound or peptide. In contrast, NIH 3T3 cells that constitutively express activated Ras in the presence of GEF have a transformed phenotype (Aronheim *et al.*, *Cell*. 78, 949-961, 1994)

[0151] In yet another embodiment, small molecules are tested for their ability to dissociate the protein complex of the invention, by an adaptation of plate agar diffusion assay described by Vidal and Endoh, *TIBS* 17, 374-381, 1999, which is incorporated herein by reference.

[0152] A further embodiment of the invention provides a method for determining a modulator of an interaction between Cbl or a portion of Cbl and a polypeptide selected

from the group consisting of APS, CAP, CrklI and C3G or a portion of said polypeptide, said method comprising:

- (i) determining the level of a protein complex selected from the group consisting of: (i) a complex comprising Cbl and APS; (ii) a complex comprising Cbl and CAP; (iii) a complex comprising Cbl and CrklI; (iv) a complex comprising Cbl and C3G; (v) a complex comprising Cbl and CAP and flotillin; and (vi) a complex comprising Cbl and CrklI and C3G in the absence of a candidate compound or candidate antibody; and
 - (ii) determining the level of said protein complex in the presence of a candidate compound or in the presence of said candidate antibody
- wherein a difference in the level of said protein complex at (i) and (ii) indicates that the candidate compound or candidate antibody is a modulator of said interaction.

[0153] This embodiment of the invention applies *mutatis mutandis* to the determination of protein complexes comprising a portion of any one or more of the protein binding partners (i.e. Cbl, APS, CAP, CrklI or C3G).

[0154] It will be understood by those skilled in the art that any one or more of the assay methods for antagonists as described herein above can be adapted for this purpose. This is because the level of the protein complex in the presence or absence of a candidate compound or antibody is related to antibody binding in the case of ELISAs, or to cell survival or growth, in the case of hybrid screens or protein recruitment assays. ELISA-based assay formats are particularly suitable for this purpose, because they are readily quantifiable, by calibrating the detection system against known amounts of a protein standard to which the antibody binds. Such quantitation is well known to the skilled person.

[0155] It is to be understood that the modulators can be antagonists or inhibitors of complex formation or stability, or alternatively, agonists or promoters of complex formation and stability.

[0156] The modulators identified using the methods described herein are useful for the therapeutic or prophylactic treatment of diseases associated with associated with Cbl function, such as, for example, modifying fat deposition or lean muscle mass or the

ratio of body fat to muscle or metabolic rate or aberrant glucose uptake or feeding behaviour. In one embodiment, the compounds are used to treat a condition selected from the group consisting of: hyperglycemia, hyperinsulinemia, obesity, adult-onset obesity, non-insulin-dependent diabetes mellitus, type II diabetes, glucose intolerance, and hypertrophy or hyperplasia of the islets of Langerhans. In another embodiment, the compounds are used for cosmetic purposes, such as, for example, by bodybuilders or persons wishing to modify their weight or body content of fat or muscle.

- 10 [0157] Accordingly, another aspect of the invention provides a method comprising administering an effective amount of a Cbl antagonist to an animal or human subject to inhibit or reduce the expression or activity of Cbl in the subject. Preferably, the subject is a subject in need of treatment, such as a subject suffering from a condition selected from the group consisting of: elevated glucose uptake, reduced appetite or dietary
15 intake, hyperglycemia, hyperinsulinemia, enhanced fat deposition or obesity, adult-onset obesity, non-insulin-dependent diabetes mellitus, type II diabetes, glucose intolerance, and hypertrophy or hyperplasia of the islets of Langerhans.

- [0158] In one embodiment, the invention also provides a method of treating a feeding
20 disorder characterized by reduced dietary intake or suppressed appetite in a subject said method comprising administering to the subject an amount of a Cbl antagonist effective to enhance the appetite or dietary intake of the subject. The method of the invention is particularly suited to the treatment of anorexia or bulimia.

- 25 [0159] In a related embodiment, the invention also provides a method of treating a feeding disorder characterized by reduced dietary intake or suppressed appetite in a subject said method comprising administering to the subject an amount of a compound that reduces expression of functional Cbl effective to enhance the appetite or dietary intake of the subject.

30

- [0160] Preferably, the compound or Cbl antagonist is a compound or antagonist identified using a screening method described herein.

[0161] To determine an appropriate dosage for treatment, data from the cell culture assays or animal studies are used, wherein a suitable dose is within a range of circulating concentrations that include the ED₅₀ of the active compound with little or no toxicity. The dosage may vary within this range depending upon the dosage form
5 employed and the route of administration utilized. For any Cbl inhibitor or antagonist used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models (e.g. any one or more of the mouse models described *supra* having genetic obesity-diabetes syndromes, such as hyperglycemia, hyperinsulinemia, and obesity) to
10 achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test Cbl inhibitor which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma maybe measured, for example, by high performance liquid chromatography.

15

[0162] In an alternative embodiment, the present invention provides for the use of a a Cbl antagonist in the preparation of a medicament for the treatment of a condition selected from the group consisting of: elevated glucose uptake, reduced appetite or dietary intake, hyperglycemia, hyperinsulinemia, enhanced fat deposition or obesity,
20 adult-onset obesity, non-insulin-dependent diabetes mellitus, type II diabetes, glucose intolerance, and hypertrophy or hyperplasia of the islets of Langerhans.

[0163] The various embodiments of the invention described herein for identifying compounds that are administered to animals are also suitably performed using
25 isolated cells that have been previously derived from the animal or readily available as isolated cells, the only requirement being that the cell possesses the required Cbl phenotype to perform the assay. In one embodiment, the isolated cells are skeletal muscle cells, cardiac muscle cells, fibroblasts or fat cells (adipocytes). In lieu of using wild-type or normal animals having no Cbl deficiency, 3T3-L1 adipocytes can be used.
30 The various embodiments apply *mutatis mutandis* to the use of such isolated cells.

[0164] The present invention is further described with reference to the following examples and the accompanying drawings.

Example 1

Methods.

- [0165] Animals were housed at the BTF facility of the Garvan Institute of Medical Research, Sydney, New South Wales, Australia. All procedures undertaken in these animals have been reviewed by the Ethics committee of the Garvan Institute. Animals were fed *ad libitum* with standard rodent chow and housed in 12 hour light/dark cycle. Body weight and food intake were monitored weekly for 12 weeks from weaning.
- 10 [0166] For determining glucose tolerance, animals were fasted overnight. Glucose (2 g/kg body weight) was administered into the intraperitoneal cavity following removal of a blood sample to test basal glucose concentration. Blood samples were taken at 15 min intervals for the following 90 min. Glucose assay was performed using the glucose oxidase method.
- 15 [0167] Fasting blood samples were also taken for measurement of circulating lipids and cytokines. Adipose depots, muscle, liver, brain were excised and weighed and frozen for subsequent biochemical analyses.
- 20 [0168] Temperature was measured using a Rectal probe (BAT-10, Physitemp) at the beginning and the end of the light cycle.
- [0169] For determining glucose transport in muscle and adipose tissue, animals were fasted overnight and whole soleus muscle or gonadal white adipose tissue explants
25 were incubated *in vitro* in the absence or presence of insulin in a Krebs Ringer buffer.
- [0170] Glucose uptake was measured using the radiolabelled 2-deoxyglucose method..
- 30 [0171] For determining adipocyte size, the adipocytes were isolated using the collagenase method and incubated overnight in 2% osmium tetroxide at 37 C. Cells were mounted on a slide and images were acquired using bright field microscopy. Cell diameter was determine using Adobe Photoshop software.

Results

[0172] Figures 1 through 6 show that Cbl-deficient mice have significantly higher body weight and dietary intake, however reduced fat deposition and smaller adipocytes, compared to otherwise isogenic non-mutant animals of the same gender. The body temperature of Cbl-deficient animals is also enhanced. Surprisingly, and in contrast to what was expected, disruption of the Cbl gene lead to enhanced basal glucose uptake into both adipocytes and muscle cells. The results are also summarized in Table 1 below.

10

Table 1
Phenotype of Cbl-deficient mice

Parameter	Phenotype in c-Cbl ^{-/-} animals compared to wild type Cbl ^{+/+} animals
Body weight	No change or slight increase in males
Food intake	Increased
Fat mass	Reduced
Adipocyte size	Reduced
Glucose tolerance	Increased
Temperature	Increased
Glucose transport into fat	Increased basal level
Glucose transport into muscle	Increased basal level
Fasting blood glucose	No change

[0173] In two independent experiments, the average body temperature of Cbl-deficient males and females increased as indicated in Table 2 below:

15

Table 2
Enhanced body temperature in Cbl-deficient animals (Average °C ± SEM)

Experiment No.	Males		Females	
	Cbl ^{+/+}	Cbl ^{-/-}	Cbl ^{+/+}	Cbl ^{-/-}
1	37.11°C ± 0.26°C	38.93°C ± 0.22°C	37.60°C ± 0.30°C	38.06°C ± 0.15°C
2	37.25°C ± 0.16°C	38.17°C ± 0.14°C	37.62°C ± 0.09°C	37.31°C ± 0.12°C

SEQUENCE LISTING

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 5 <120> Methods of treatment of feeding disorders and disorders of
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 30 Val Asp Lys Lys Met Val Glu Lys Cys Trp Lys Leu Met Asp Lys Val
 50 55 60
 Val Arg Leu Cys Gln Asn Pro Asn Val Ala Leu Lys Asn Ser Pro Pro
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 100 105 110
 Phe Arg Val Phe Met Glu Asn Leu Met Lys Lys Thr Lys Gln Thr Ile
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 35 40 45
 10 Val Asp Lys Lys Met Val Glu Lys Cys Trp Lys Leu Met Asp Lys Val
 50 55 60
 Val Arg Leu Cys Gln Asn Pro Asn Val Ala Leu Lys Asn Ser Pro Pro
 15 65 70 75 80
 Tyr Ile Leu Asp Leu Leu Pro Asp Thr Tyr Gln His Leu Arg Thr Val
 85 90 95
 20 Leu Ser Arg Tyr Glu Gly Lys Met Glu Thr Leu Gly Glu Asn Glu Tyr
 100 105 110
 Phe Arg Val Phe Met Glu Asn Leu Met Lys Lys Thr Lys Gln Thr Ile
 115 120 125
 25 Ser Leu Phe Lys Glu Gly Lys Glu Arg Met Tyr Glu Glu Asn Ser Gln
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 Pro Arg Arg Asn Leu Thr Lys Leu Ser Leu Ile Phe Ser His Met Leu
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 Gly Glu Lys Thr Ile Val Pro Trp Lys Ser Phe Arg Gln Ala Leu His
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 Ile Phe Thr Arg Leu Phe Gln Pro Trp Ser Ser Leu Leu Arg Asn Trp
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 260 265 270
 Asp Glu Val Lys Ala Arg Leu Gln Lys Phe Ile His Lys Pro Gly Ser
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 290 295 300

Tyr Val Thr Ala Asp Gly Asn Ile Leu Gln Thr Ile Pro His Asn Lys
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 5 Pro Leu Phe Gln Ala Leu Ile Asp Gly Phe Arg Glu Gly Phe Tyr Leu
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 Phe Pro Asp Gly Arg Asn Gln Asn Pro Asp Leu Thr Gly Leu Cys Glu
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 10 Pro Thr Pro Gln Asp His Ile Lys Val Thr Gln Ile Cys Ala Glu Asn
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 15 Asp Lys Asp Val Lys Ile Glu Pro Cys Gly His Leu Met Cys Thr Ser
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 Pro Lys Val Pro Val Ala Thr Pro Asn Pro Gly Asp Pro Trp Asn Gly
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 595 600 605

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 Glu His Pro Lys Ile Lys Pro Ser Ser Ser Ala Asn Ala Ile Tyr Ser
 645 650 655
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 740 745 750
 30 Pro Glu Glu Ser Glu Asn Glu Asp Asp Gly Tyr Asp Val Pro Lys Pro
 755 760 765
 Pro Val Pro Ala Val Leu Ala Arg Arg Thr Leu Ser Asp Ile Ser Asn
 770 775 780
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 Phe Asn Glu Gly Ser Gln Val Pro Glu Arg Pro Pro Lys Pro Phe Pro
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 55

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5

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 35 40 45

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 50 55 60

20 Lys Val Val Arg Leu Cys Gln Asn Pro Lys Leu Ala Leu Lys Asn Ser
 65 70 75 80

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DATED this SIXTEENTH day of DECEMBER, 2002

10 GARVAN INSTITUTE OF MEDICAL RESEARCH

by *FB RICE & CO*
Patent Attorneys for the applicant

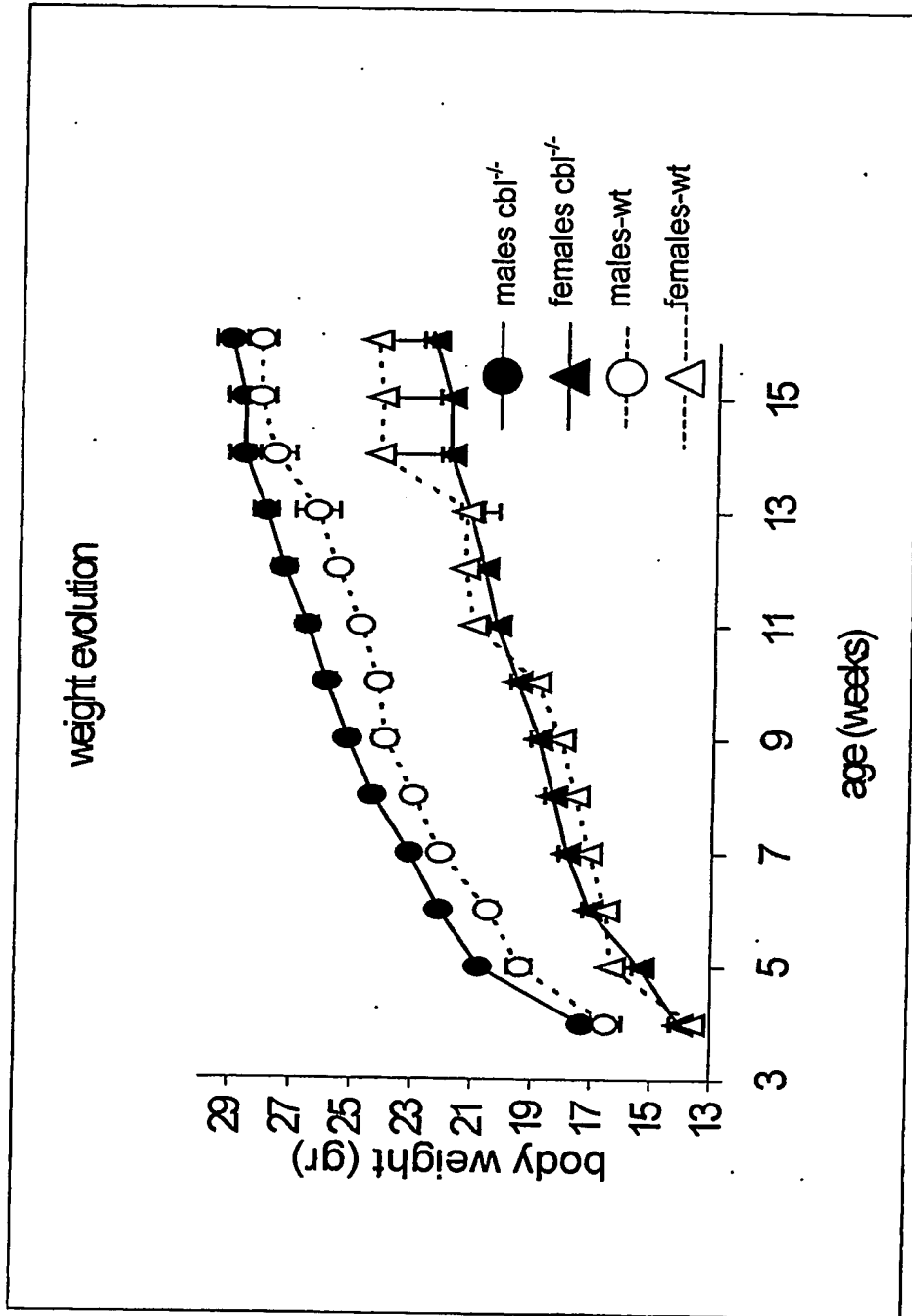


FIGURE 1

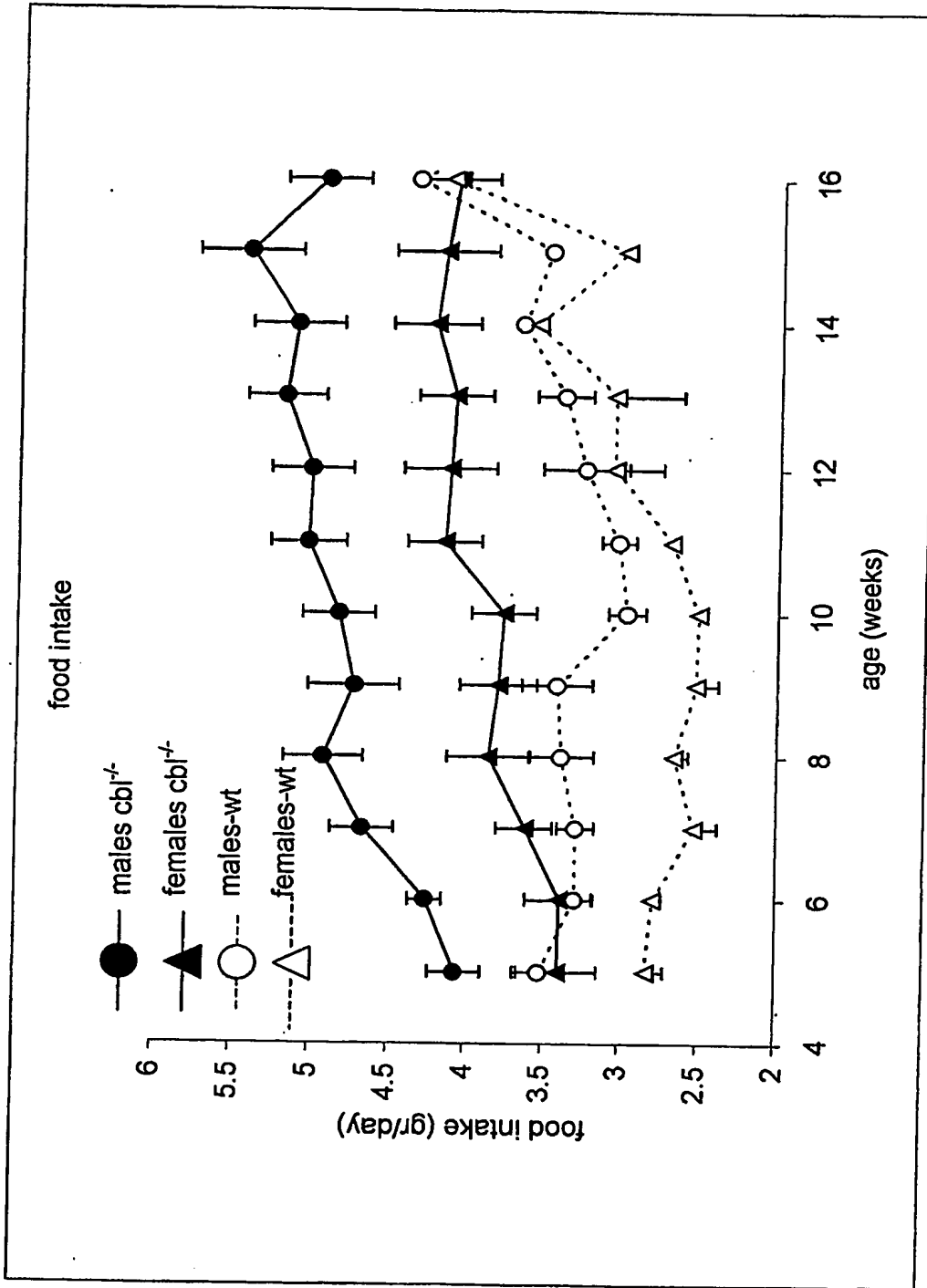


FIGURE 2

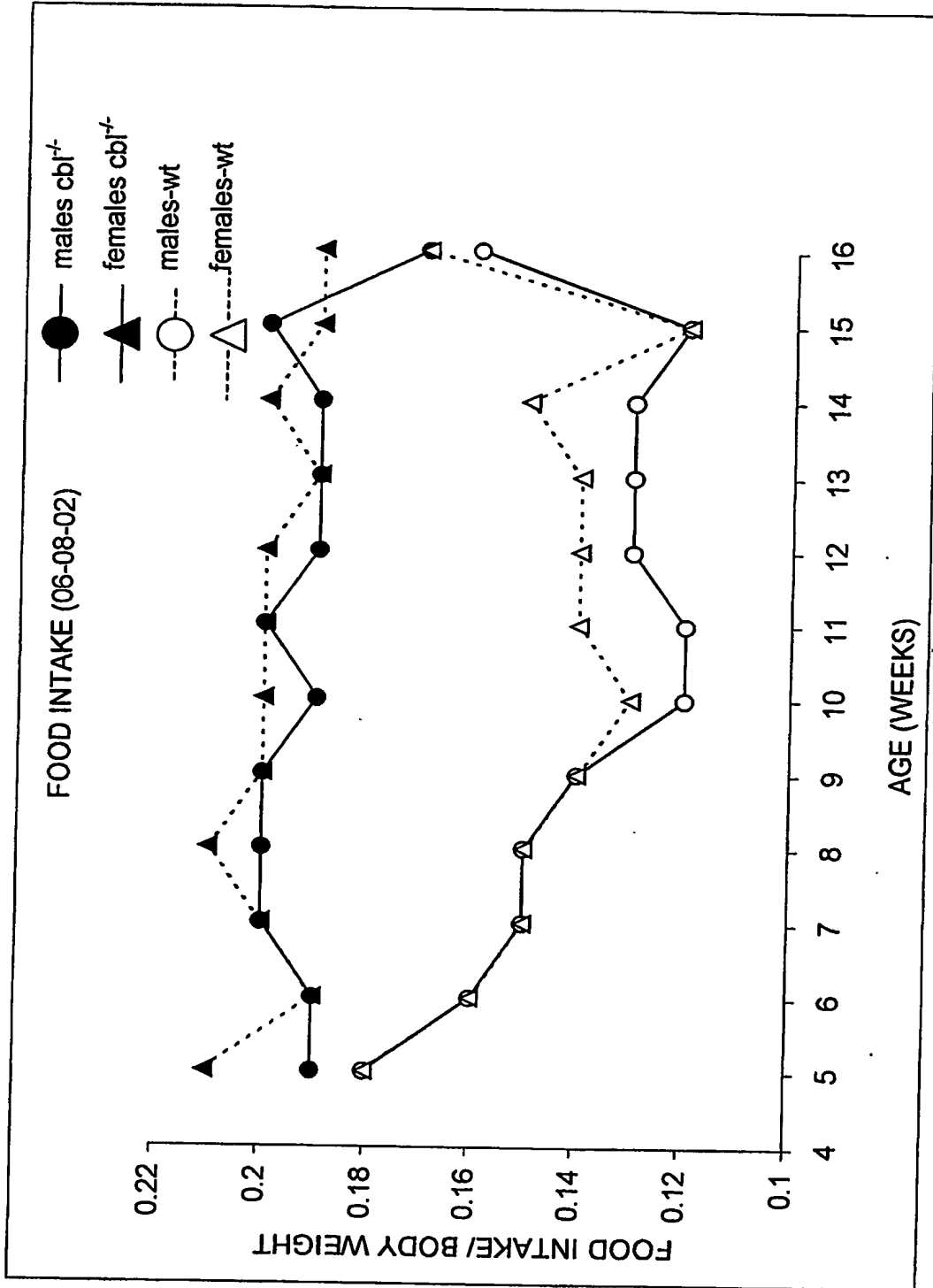


FIGURE 3

TISSUE WEIGHT

Wild Type

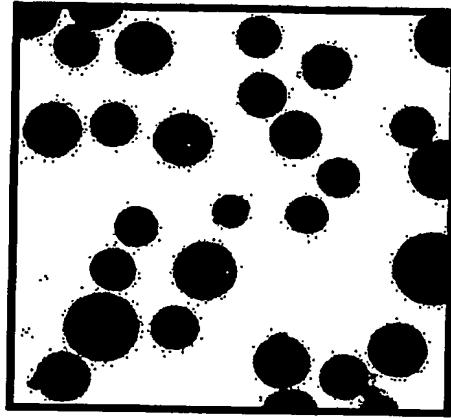
AVG	30.4	0.543	0.141	0.204	100	1.79	0.46	0.67
SEM	0.54	0.129	0.002	0.011		0.46	0.01	0.02
	BW	WAT	BAT	QUAD	BW	WAT	BAT	QUAD

4/6

Cbl-deficient

AVG	30.00	0.226	0.073	0.206	100.00	0.75	0.24	0.69
SEM	0.95	0.021	0.005	0.013		0.07	0.01	0.04
	BW	WAT	BAT	QUAD	BW	WAT	BAT	QUAD

FIGURE 4



Cbl-deficient



Wild Type

FIGURE 5

ADIPOCYTE SIZE MALES

	diameter (μm)		VOLUME (pl/cell)		Lipid content (ng/cell)	
	AVG	SEM	AVG	SEM	AVG	SEM
WT	84.91	1.57	240.28	4.45	219.86	4.07
KO	49.44	0.90	82.43	1.25	75.42	1.14
KO 500	56.82	1.23	72	1.56	65.88	1.43

ADIPOCYTE SIZE FEMALES

	diameter (μm)		VOLUME (pl/cell)		Lipid content (ng/cell)	
	AVG	SEM	AVG	SEM	AVG	SEM
WT	80.48	2.32	204.6	5.9	187.3	5.4
KO	55.21	1.77	66.05	2.12	60.92	1.96

FIGURE 6